FAT - SUDAN BLACK B- PROPYLENE GLYCOL

PURPOSE: For the demonstration of fat.

PRINCIPLE: Sudan Black is slightly basic dye and will combine with acidic groups in compound lipids, thus staining phospholipids also. An alternative stain to the Sudan Black B stain.

CONTROL: Use a positive control of a fat smeared slide, and a negative control slide of a paraffin processed tissue, such as lung.

FIXATIVE: 10% formalin.

TECHNIQUE: Cut frozen tissue sections 10µ.

EQUIPMENT: Cryostat, coplin jars. (Making stain, stir plate, filter paper, fritted glass filter, and vacuum) and a 60℃ oven. Rinse all glassware in DI water.

REAGENTS:

Propylene Glycol:
Place in two coplin jars, label #1 and #2, can be reused.

CAUTION: Avoid contact and inhalation.

85% Propylene Glycol:

Propylene glycol	85.0 ml
Distilled water	15.0 ml

CAUTION: Avoid contact and inhalation.

Hematoxylin:
Commercial Gill-3

Glycerin Jelly

Sudan Black B/Propylene:

Sudan Black B	0.7 gm
Propylene glycol	100.0 ml

Dissolve sudan black in propylene glycol, slowly, while stirring. Heat to 100℃, but not over 110℃, for a few minutes, stirring constantly. Filter through Whatman #2 filter paper. Cool, and filter again through a frittered glass filter of medium porosity with suction. Store in a 60℃ oven. Solution stable for 1 year.

CAUTION: Avoid contact and inhalation.

SAFETY: Wear gloves, goggles and lab coat. Avoid contact and inhalation. Propylene glycol; mild skin and eye irritant. Cumbustible.

PROCEDURE:

1. Pick-up frozen sections on clean glass slides if fresh, albuminized slides if fixed.
2. Fix slides in 10% formalin if fresh.
3. Wash well it tap, rinse in distilled, drain off excess water.
4. Propylene glycol, two changes, 5 minutes each.
5. Sudan Black, 7 minutes, agitate.
6. 85% Propylene glycol, 3 minutes.
7. Rinse in distilled water.
8. Nuclear Fast Red, 3 minutes.
9. Wash in water.
10. Wash in tap water, rinse in distilled.
11. Mount with aqueous mounting media, Glycerin Jelly.

RESULTS:

Fat	blue-black
Nuclei	red

REFERENCE:

Carson, F, Histotechnolog: A Self-Instructional Text, 1st Ed,1990, pp161-62, ASCP Press

Crookham,J, Dapson,R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

Prepared:__________________________ By: __________________

Approved:__________________________ By:__________________

Downloaded from WebPath: Internet Pathology Laboratory
http://www-medlib.med.utah.edu/WebPath/webpath.html

PROCEDURE CARD

FAT - SUDAN BLACK B- PROPYLENE GLYCOL

CONTROL: Use a positive control of a fat smeared slide, and a negative control slide of a paraffin processed tissue, such as lung.

FIXATIVE: 10% formalin.

TECHNIQUE: Cut frozen tissue sections 10µ.

PROCEDURE:

1. Pick-up frozen sections on clean glass slides if fresh, albuminized slides if fixed.
2. Fix slides in 10% formalin if fresh.
3. Wash well it tap, rinse in distilled, drain off excess water.
4. Propylene glycol, two changes, 5 minutes each.
5. Sudan Black, 7 minutes, agitate.
6. 85% Propylene glycol, 3 minutes.
7. Rinse in distilled water.
8. Nuclear Fast Red, 3 minutes.
9. Wash in water.
10. Wash in tap water, rinse in distilled.
11. Mount with aqueous mounting media, Glycerin Jelly.

RESULTS:

Fat	blue-black
Nuclei	red

Propylene Glycol:
Place in two coplin jars, label #1 and #2, can be reused.

CAUTION: Avoid contact and inhalation.

85% Propylene Glycol:

Propylene glycol	85.0 ml
Distilled water	15.0 ml

CAUTION: Avoid contact and inhalation.

Hematoxylin:
Commercial Gill-3

Glycerin Jelly

Sudan Black B/Propylene:

Sudan Black B	0.7 gm
Propylene glycol	100.0 ml

Dissolve sudan black in propylene glycol, slowly, while stirring. Heat to 100℃, but not over 110℃, for a few minutes, stirring constantly. Filter through Whatman #2 filter paper. Cool, and filter again through a frittered glass filter of medium porosity with suction. Store in a 60℃ oven. Solution stable for 1 year.

CAUTION: Avoid contact and inhalation.

SUDAN BLACK B/PROPYLENE:

Sudan Black B	0.7 gm
Propylene glycol	100.0 ml

Dissolve sudan black in propylene glycol, slowly, while stirring. Heat to 100℃, but not over 110℃, for a few minutes, stirring constantly. Filter through Whatman #2 filter paper. Cool, and filter again through a frittered glass filter of medium porosity with suction. Store in a 60℃ oven. Solution stable for 1 year.

CAUTION: Avoid contact and inhalation.

DATE:______________________________

TECH:______________________________

EXPIRATION:_______________________

PROPYLENE GLYCOL #1

DATE:__________________

TECH:__________________

PROPYLENE GLYCOL #2

DATE:__________________

TECH:__________________